Evolution and development of orchid flowers and fruits

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Chapter 2

Floral development: Lip formation in orchids unraveled

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Most orchid flowers have an enlarged median petal, the 'lip', which plays a crucial role in attracting pollinators. The existence and appearance of this organ is due to the presence of specific protein complexes involved in floral development, which are differentially expressed in orchid species with more or less pronounced lips. Attracting pollinators for reproduction is a major challenge for plant species. As plants generally cannot move, they evolved flowers to attract pollinators by employing dazzling colors, alluring odors and tactile cues.

Flowers are composed of several whorls. The outer whorl is made up of sepals that generally serve as protection, while the second whorl consists of petals involved in pollinator attraction. Sepals and petals together form the perianth enfolding the male and female reproductive organs. Orchids are unusual in that their floral morphology includes petal-like sepals, brightly colored and ornamented, containing special surface ornaments to attract insects. Furthermore, the median petal is transformed into a lip that acts as the main attractive organ (**Figure 1**). Hsu *et al.* (2015) described how competition between two protein complexes determines the development of this peculiar lip organ. This greatly extends our understanding of the mechanisms leading to the diverse forms of orchid flowers.

Figure 1. Flower of *Ophrys splendida* with *Andrena* male. The bee clings to the lip, which mimics a female bee in appearance, fragrance and physical touch. The petals and sepals are bent backwards, ensuring maximal contact between the yellow pollen packages on the bee's head and the female reproductive part of the flower that it attempts to mate with [Photo by Jean Claessens].

The only subfamily of orchids without a pronounced lip is the most basal and least diverse lineage. Therefore the evolution of a lip clearly triggered speciation and can be seen as a key innovation. To dissect the molecular basis of lip development, studies have been conducted on the MADS-box genes involved in promoting petal identity, which revealed a number of duplication events that occurred after orchids started to diversify, more than 60 million years ago (Ramirez *et al*., 2007). These duplication events appear to have generated copies of MADS-box genes with new functions. Hsu *et al.* (2015) comprehensively examined the expression of all known MADS-box genes of the A, B and E classes in the flowers of certain orchid species. They found that different copies of duplicated MADS-box genes (*AP3* in the B-class and *AGL6* in the E-class) showed different tissue-specific or tissue-biased expression (Hsu *et al*., 2015).

Based on this expression pattern, Hsu *et al.* (2015) proposed the 'Perianth code' to link gene expression with petal/lip identity. According to the perianth code, two protein complexes, the 'L' complex and the 'SP' complex, each produced by four MADS-box genes, compete to promote the formation of lip and petals, respectively. Fluorescence resonance energy transfer (FRET) analyses support the existence of the two complexes. The relative expression of the two determines to what extent, in some orchids, the lip is more or less differentiated from the sepals and petals.

Hsu *et al.*(2015) validated the perianth code in peloric floral mutants. Such a mutant of *Oncidium* 'Gower Ramsey' exhibits complete transformation of petals into lip-like structures. As predicted, it lost the expression of the SP complex and only expressed the L complex in the petals. Similarly, the peloric mutants displaying partial petal-to-lip conversion showed reduced SP expression but elevated L complex expression in the petals. The researchers further tested the perianth code in *Oncidium* species with different types of perianth conversion. Species with lip-like petals or petal-like lips showed co-existence of the SP and L units or absence of both complexes in petals or lips. Hence, the antagonism between the two complexes successfully explains the diverse flower forms across multiple orchid species and cultivars.

Expression analyses only provide part of the evidence for this hypothesis. To substantiate the function of the perianth code, Hsu *et al.* (2015) manipulated the balance between the two complexes using virus induced gene silencing (VIGS), a technique employing agrobacterium strains containing a modified virus for silencing specific genes. The *OAGL6-2* gene encoding a component of the L complex was down regulated by VIGS in *Oncidium* and *Phalaenopsis* orchids, resulting in the conversion of lips to sepal/petal structures in both scenarios. The resulting phenotypes represent solid functional support for the perianth code.

The ABCDE model of floral development describes interactions between specific MADS-box proteins determining the identities of flower organs (Coen and Meyerowitz, 1991). One protein complex consisting of class A and class E MADS-box proteins specifies sepals, whereas another composed of A, B and E class proteins controls petals. According to the floral quartet model, a combination of four proteins forms whorl-specific complexes (Theissen and Saedler, 2001). These complexes function by either activating or silencing target genes during floral development. Both models apply to many flowering plant families. However, for the highly specialized sepals, petals and lip in the first and second whorl of most orchid flowers, the perianth code model (Hsu *et al*., 2015) proves to be a better analogy.

Hsu *et al.* (2015) have shown that two protein complexes produced by A/E and B class genes determine which organs develop in the orchid perianth. When the balance between both complexes is shifted by partially silencing the one involved in lip formation, only sepal- and petal-like organs develop. This experiment nicely shows that during orchid evolution two copies of duplicated developmental genes acquired a new function by specifically promoting lip identity.

In 1877, Charles Darwin published an extensive overview of highly specialized organs in orchid flowers and speculated about their role in maximizing pollination success. It is now assumed that the evolution of these organs provided a strong selection advantage allowing one-third of all orchid species, distributed over many unrelated clades, to be fertilized without offering any food reward to their pollinators (Cozzolino and Widmer, 2005). This makes attraction very challenging as pollinators quickly learn to avoid such flowers. The discovery of the protein complexes involved in lip formation in orchid flowers is intriguing, and provides an important step towards fully understanding how orchids continue to lure pollinators despite the lack of a material benefit. Unraveling the genetic basis of other highly specialized floral organs will undoubtedly provide further insights into the contribution of duplicated and neo-functionalized developmental genes to the evolutionary arms race between orchids and their pollinators.

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